

CELL DIVISION-INDUCING SUBSTANCES IN THE LIQUID ENDOSPERM OF *HYPHAENE NATALENSIS*

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ABSTRACT

The presence of at least two cell division-inducing compounds in the liquid endosperm of *Hyphaene natalensis* was indicated by results obtained in the soybean bioassay. Most of the activity is attributable to a butanol-soluble compound that co-elutes with zeatin riboside on a Sephadex LH-20 column.

UITTREKSEL

SELDELINGS-STIMULEERDERS IN DIE VLOEIBARE ENDOSPERM VAN *HYPHAENE NATALENSIS*

Met behulp van die sojaboonkallus-biotoets is gevind dat die vloeibare endosperm van *Hyphaene natalensis* minstens twee seldelings-stimuleerders bevat. Die meeste van die aktiwiteit is toe te skryf aan 'n butanol-oplosbare verbinding met dieselfde elueringspatroon, op Sephadex LH-20, as zeatiëneribosied.

INTRODUCTION

Hyphaene natalensis Kunze, commonly known as the ilala palm or ivory nut palm grows along the Natal coast and also in the inland lowveld areas of Tongaland in northernmost Natal. The fruits which are borne on branched inflorescences, are pear-shaped and remain on the tree for a long time, usually taking two or more years to reach maturity. In the early stages of development the endosperm, as in other palm fruits, is liquid. In the case of *Cocos nucifera* L. this liquid endosperm is extremely rich in growth promoting substances e.g. cytokinins (Letham, 1968) and for this reason is frequently used as an additive in plant tissue culture experiments. The present study was conducted to determine whether the liquid endosperm of *Hyphaene natalensis* contains similar substances.

EXPERIMENTS AND RESULTS

The fruits of *Hyphaene natalensis* collected in Tongaland during April, 1973, and used in the present investigation, each yielded between three and four millilitres of liquid endosperm ("milk"). In order to establish whether this milk contained any cell division-inducing substances a series of dilutions, ranging from 0.01%–10% of the culture medium was assayed for cell division activity

with the soybean bioassay (Miller, 1965). For comparison a series of dilutions of coconut milk was assayed simultaneously. Figure 1 shows that ilala milk does contain substances that enhance the growth of soybean callus. The concentration of these substances is apparently much lower than in coconut milk as 0,1% coconut milk gave a better response than any of the concentrations of ilala milk used. With ilala milk the highest activity was recorded when it constituted 5% of the culture medium. Higher concentrations depressed growth, probably as a result of inhibiting or interfering substances which offset the effect of the growth promoting compounds. That phenolic compounds could be involved is suggested by the fact that the culture medium containing 7,5% and 10% ilala milk respectively turned brown upon autoclaving. In order to eliminate interfering compounds and obtain more information about the nature of the cell division-inducing compounds involved, 600 ml of ilala milk was concentrated to 100 ml under vacuum at 40°C. The concentrated solution was partitioned against water-saturated n-butanol at pH 7,0 in order to separate the butanol-soluble free base and riboside cytokinins from the water-soluble cytokinin ribotides. The resultant aqueous and butanol extracts were reduced to dryness *in vacuo* at 35°C, taken up in a small volume of 35% ethanol and then streaked onto Whatman 3 MM chromatography paper. Chromatograms were developed in *iso*-propanol: ammonia:water (10:1:1 v/v), dried and divided into 10 equal R_f zones. The equivalent of 300 ml milk was assayed in 40 ml medium, and cultured for a period of 28 days.

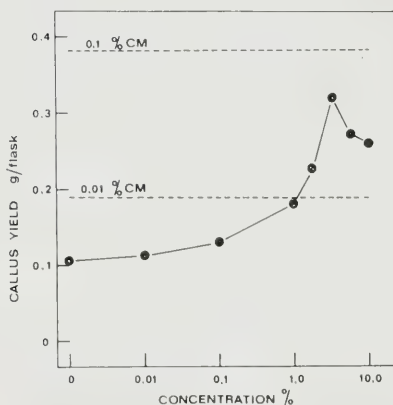


FIG. 1.

Relationship between the concentration of ilala milk and the growth of soybean callus. CM = coconut milk.

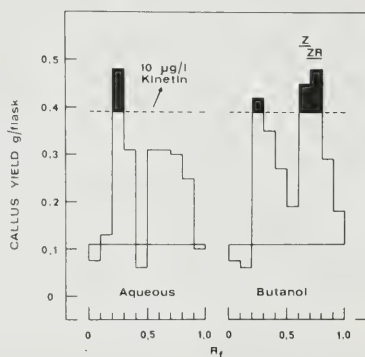


FIG. 2.

Soybean bioassay of aqueous and butanol extracts of 300 ml equivalents of ilala milk. Solvent: *iso*-Propanol: ammonia: water (10:1:1 v/v). The position of zeatin (Z) and zeatin riboside (ZR) when run as markers, are indicated.

Substances that enhance the growth of soybean callus were present in both the aqueous and butanol extracts (Fig. 2). In the aqueous extract the major peak of activity was found between Rf 0,2–0,3. Two peaks of activity were found in the butanol extract. One was slow-moving and occurred in the same region of the chromatogram as the active compounds in the aqueous extract. This activity was in all probability due to water-soluble cytokinins passing into the butanol during partitioning. The second peak of activity occurred between Rf 0,6–0,8, that is in the region where cytokinin ribosides and their free bases are expected to occur. In agreement with the paper chromatographic results, fractionation of a butanol extract, from 500 ml equivalent of ilala milk, on Sephadex LH-20 (Van Staden, Webb and Wareing, 1972) with 35% ethanol, resulted in two major peaks of activity (Fig. 3). Activity in the bioassays was regarded as being significant when growth higher than that recorded for 10 $\mu\text{g/l}$ kinetin equivalents was recorded. Most of the activity is apparently due to a compound which is similar, or has the same elution volume on Sephadex, as zeatin riboside. The second peak eluted from the column very rapidly (200–240 ml) and did not co-chromatograph with available naturally-occurring cytokinin markers. Indications are that activity in this region of the chromatogram could be due to the

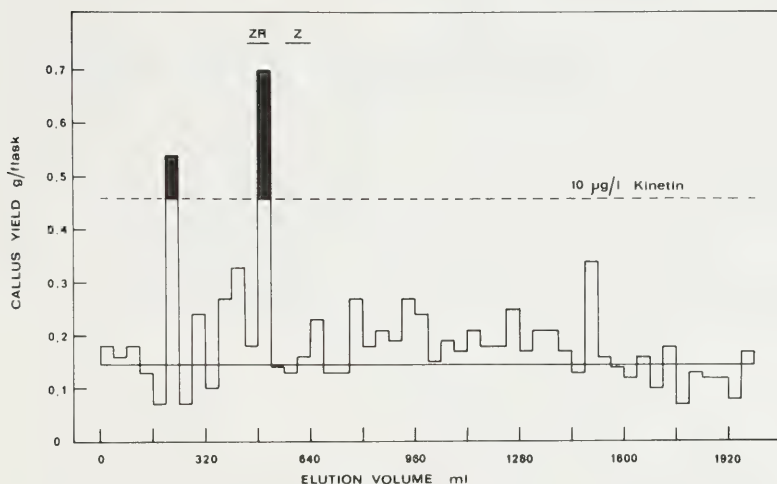


FIG. 3.

Soybean bioassay of a butanol extract of ilala milk (500 ml) after partition chromatography on Sephadex LH-20. The position of zeatin riboside (ZR) and zeatin (Z) when run as markers are indicated.

presence of cytokinin ribotides. This is substantiated by the fact that elution of the peak from aqueous extracts after paper chromatography (Rf 0,2–0,3 in Fig. 2), and its subsequent fractionation of Sephadex LH-20, yielded one peak of activity with the same elution characteristics.

DISCUSSION

Both water and butanol-soluble cytokinin-like substances were present in the liquid endosperm of the ilala palm. Most of the activity is apparently due to the presence of a compound which is similar or has the same chromatographic characteristics as zeatin riboside. This is in agreement with results obtained for the coconut palm in that the major active compound in its liquid endosperm was identified as 9- β -D-ribofuranosylzeatin (Letham, 1968). The total cytokinin activity recorded for ilala milk however, appears to be more than 50 times less than that recorded for coconut milk. This finding was substantiated further by additional paper and column chromatographic techniques (unpublished data). The low cell division activity, presence of large amounts of phenolic substances, and the low yield of milk per fruit suggests that it would not be feasible to use ilala milk as an additive to induce cell division in plant tissue culture experiments.

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